

IN THE SPECIFICATION:

Please amend page 1, lines 1, 3, 6, 17, 23-25 and 28 as follows:

[description]

[Title of the Invention]

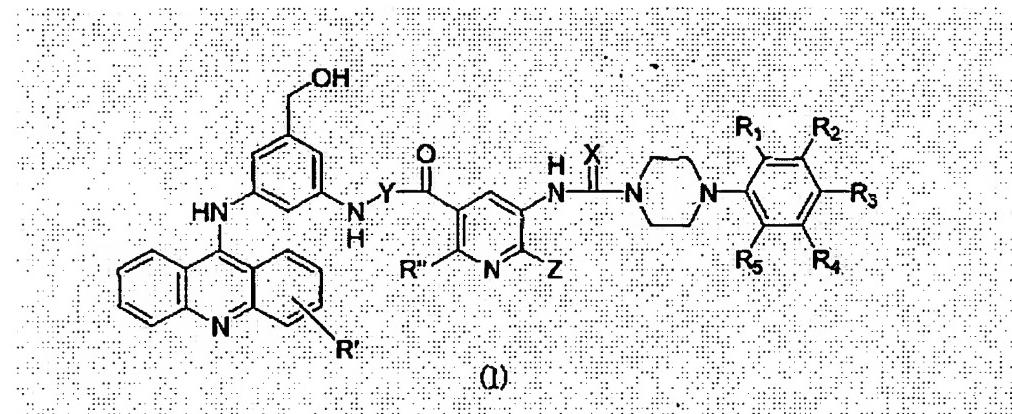
~~9-Aminoacridine derivatives and process for the preparation thereof~~

9-AMINOACRIDINE DERIVATIVES AND PROCESS FOR THE PREPARATION
THEREOF

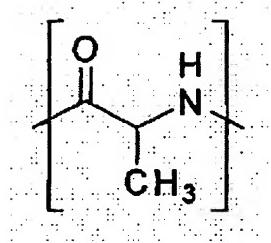
[Technical Field] **BACKGROUND OF THE INVENTION**

1. Field of the Invention

The present invention relates to a new 9-aminoacridine derivative of the general formula (I)



wherein Y is ~~zero~~ a bond or



(wherein X is oxygen or sulfur, R₁, R₂, R₃, R₄ and R₅ are independently hydrogen, halogen, nitro, amino, hydroxy, C₁-C₄ lower alkylhydroxy, C₁-C₄ lower alkylamino, C₁-C₈ alkyl or C₁-C₄ lower alkoxy, R' and R'' are independently C₁-C₈ alkyl or C₁-C₄ lower alkoxy, and Z is C₁-C₄ lower alkyl, C₁-C₄ lower alkoxy or C₁-C₄ lower alkylamino.

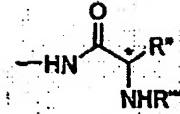
In the above definitions, C₁-C₄ lower alkyl means straight or branched alkyl groups such as methyl, ethyl, propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl or the like.

Please amend page 2, line 7 and 26 as follows:

~~[Back ground of the technology]~~

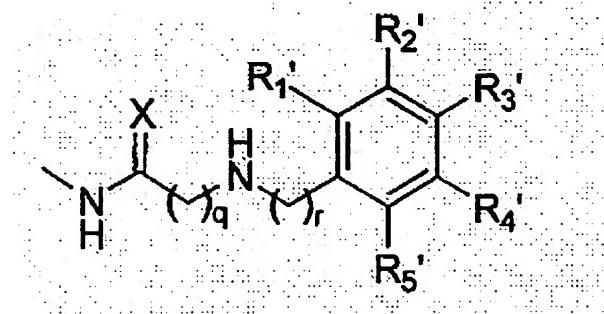
2. Description of the Prior Art

(wherein X is oxygen or sulfur, R₁, R₂, R₃, R₄ and R₅ are independently hydrogen, halogen, nitro, amino, hydroxy, C₁-C₄ lower alkylhydroxy, C₁-C₄ lower alkylamino, C₁-C₈ alkyl, C₁-C₄ lower alkoxy or C₁-C₄ lower alkyloxycarbonyl and m and n are independently an integer of 0, 1 or 2.), R₆, R₇, R₈ and R₉ are independently C₁-C₈ alkyl or C₁-C₄ lower alkoxy, and Y is hydrogen, amino, -N=CHR' (wherein R' is hydrogen, benzyl,



C1-C8 alkyl or C1-C6 lower alkylamino),

(wherein R'' is hydrogen, benzyl, C1-C8 alkyl or C1-C6 lower alkylamino, and R''' is hydrogen, benzyl, C1-C8 alkyl or amino protecting group) or



(wherein, X is as defined above, R1', R2', R3', and R4' and R5' are independently hydrogen, halogen, nitro, amino, hydroxy, C1-C4 lower alkylhydroxy, C1-C4 lower alkylamino, C1-C8 alkyl, C1-C4 lower alkoxy or C1-C4 lower alkyloxycarbonyl, and q and r are independently an integer of 0, 1 or 2) or its pharmaceutically acceptable salt, and process for the preparation thereof.

Please amend page 3, line 27 as follows:

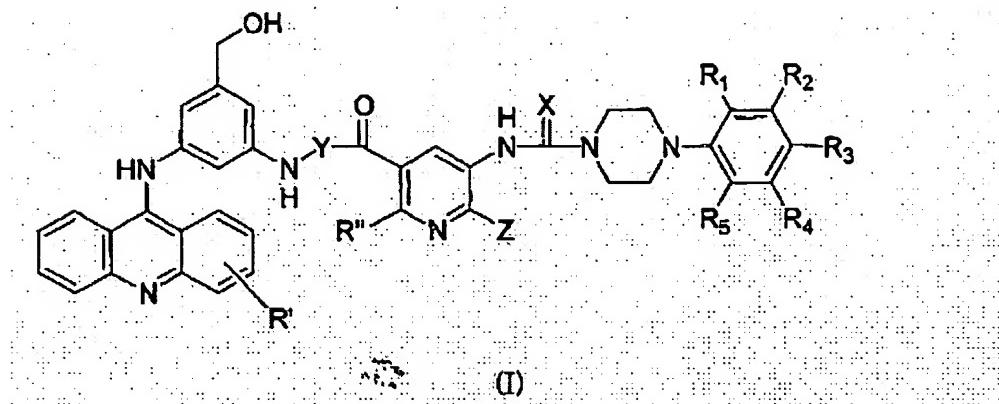
[Detailed description of the invention]

SUMMARY OF THE INVENTION

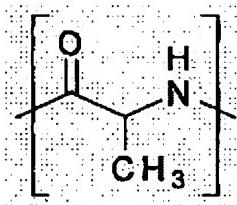
Please amend page 4, line 12 as follows:

The inventors had studied for a long time to find new compounds having intensive antitumor activities. As a result, the inventors have found out that the compounds of the general formula

(I), or acid addition salts thereof as defined above have not only prominent antitumor activities but also very low toxicities.



wherein Y is zero a bond or



(wherein X is oxygen or sulfur, R₁, R₂, R₃, R₄ and R₅ are independently hydrogen, halogen, nitro, amino, hydroxy, C₁-C₄ lower alkylhydroxy, C₁-C₄ lower alkylamino, C₁-C₈ alkyl or C₁-C₄ lower alkoxy, R' and R'' are independently C₁-C₈ alkyl or C₁-C₄ lower alkoxy, and Z is C₁-C₄ lower alkyl, C₁-C₄ lower alkoxy or C₁-C₄ lower alkylamino.

Please amend page 5, line 27 as follows:

Vehicles used in formulating pharmaceutical preparations containing the compound of the general formula (I) as an active ingredient are sweetening agents, binding agents, dissolving agents, aids for dissolution, wetting agents, emulsifying agents, isotonic agents, adsorbents, degrading agents, antioxidants, preservatives, lubricating agents, fillers, perfume or the like; for

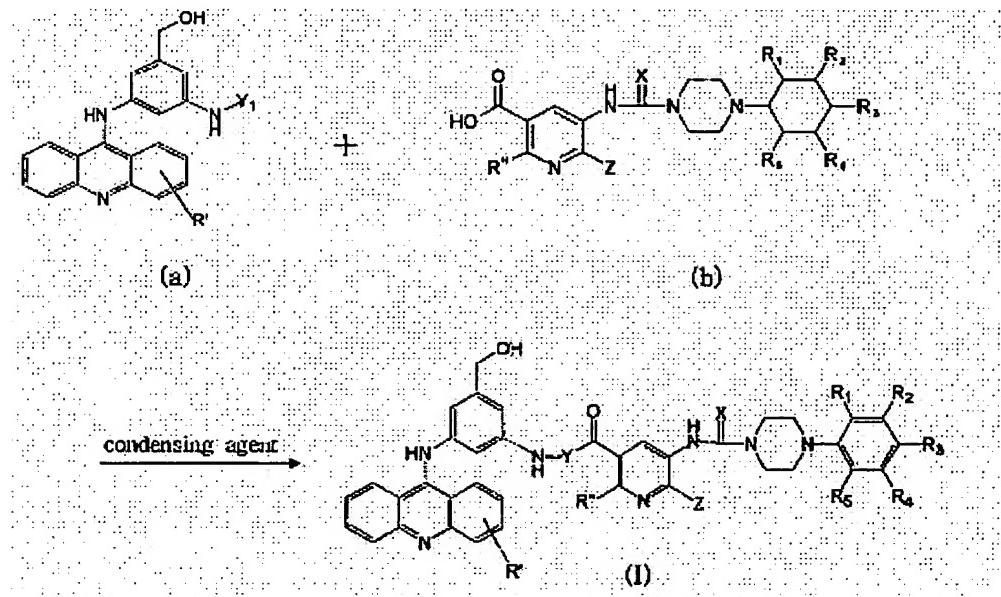
example may include lactose, dextrose, sucrose, mannitol, sorbitol, cellulose, glycine, silica, talc, stearic acid, stearin, magnesium stearate, calcium stearate, magnesium aluminum silicate, starch, gelatine, tragacanth gum, glycine, silica, alginic acid, sodium alginate, methyl cellulose, sodium carboxy methyl cellulose, agar, water, ethanol, polyethyleneglycol, polyvinyl pyrrolidone, sodium chloride, potassium chloride, orange essence, strawberry essence and vanilla aroma.

Please amend page 6, line 5 as follows:

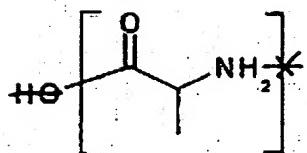
Scheme I DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT(S)

The compound of the general formula (I) according to the present invention may be prepared by following schemes I, II.

Scheme I



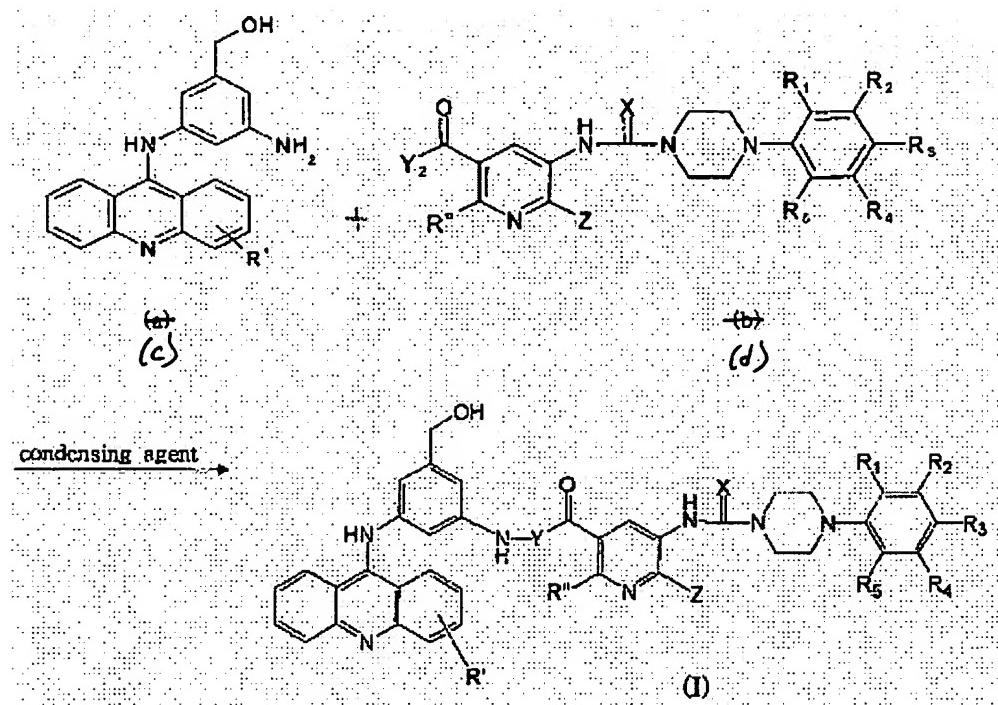
wherein R₁, R₂, R₃, R₄ and R₅, R'', X, Y and Z are as defined above and



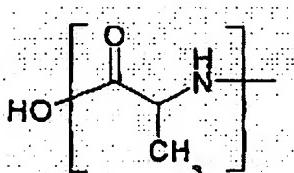
Y_1 is hydrogen or the group of

Please amend page 7, line 23 as follows:

Scheme II



wherein R_1 , R_2 , R_3 , R_4 , R_5 , R' , R'' , X , Y and Z are as defined above and



Y_2 is -OH or the group of

Please amend page 20, line 19 as follows:

Example 19

4-(3,5-dimethylphenyl)piperazine-1-carboxylic acid {5-[3-(acridine-9-yl-amino)-5-hydroxymethylphenylcarbamoyl]-6-ethyl-2-methoxy-pyridine-3-yl}amide

The same reaction procedure to the example 17 18 were carried out using 2-ethyl-5-{{[4-(3,5-dimethylphenyl)-piperazine-1-carbonyl]-amino}-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 69.5%
m.p. : 178~180°C

¹H NMR(DMSO-d₆) : 1.89(3H,t), 2.28(6H,s), 2.70(2H,q), 3.31(4H,m),
3.71(4H,m), 3.99(3H,s), 4.51(2H,s), 5.28(1H,t),
6.69(1H,s), 6.89(1H,s), 7.08(1H,s), 7.53(2H,m),
7.71(1H,s), 7.87(1H,s), 8.04(3H,m), 8.18(3H,m),
8.37(2H,m), 10.46(1H,s), 11.55(1H,s),
12.28(1H,s), 14.88(1H,s)

Please amend page 21, lines 6 and 23 as follows:

Example 20

4-(3,5-dimethoxyphenyl)-piperazine-1-carboxylic acid {5-[3-(acridine-9-yl-amino)-5-hydroxymethylphenylcarbamoyl]-6-ethyl-2-methoxy-pyridine-3-yl}amide.

The same reaction procedure to the example 17 18 were carried out using 2-ethyl-5-{{[4-(3,5-dimethoxyphenyl)-piperazine-1-carbonyl]-amino}-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 70.2%
m.p. : 170~172°C

¹H NMR(DMSO-d₆) : 1.25(3H,t), 2.84(2H,q), 3.24(4H,m), 3.66(4H,m),
3.76(6H,s), 4.04(3H,s), 4.58(2H,s), 5.28(1H,t),
6.02(1H,s), 6.08(1H,s), 6.90(1H,s), 7.26(2H,m),
7.34(1H,m), 7.42(1H,m), 7.58(1H,s), 7.62(2H,m),
7.75(2H,m), 7.88(1H,d), 8.03(2H,m), 8.23(2H,m),
8.37(1H,s), 10.06(1H,s)

Example 21

4-(3,5-difluorophenyl)-piperazine-1-carboxylic acid {5-[3-(acridine-9-yl-amino)-5-hydroxymethylphenylcarbamoyl]-6-ethyl-2-methoxy-pyridine-3-yl}amide

The same reaction procedure to the example 17 18 were carried out using 2-ethyl-5-{{[4-(3,5-difluorophenyl)-piperazine-1-carbonyl]-amino}-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 68.6%
m.p. : 184~186°C

¹H NMR(DMSO-d₆) : 1.24(3H,t), 2.79(2H,q), 3.31(4H,m), 3.59(4H,m),
3.98(3H,s), 4.47(2H,s), 5.19(1H,t), 6.53(2H,m),
6.70(2H,d), 7.07(1H,m), 7.38(3H,m), 7.51(3H,m),
8.05(3H,m), 10.23(1H,s), 10.93(1H,s)

Please amend page 22, lines 7 and 24 as follows:

Example 22

4-(3,5-dichlorophenyl)-piperazine-1-carboxylic acid {5-[3-(acridine-9-yl-amino)-5-hydroxymethylphenylcarbamoyl]-6-ethyl-2-methoxy-pyridine-3-yl}amide.

The same reaction procedure to the example 17 18 were carried out using 2-ethyl-5-{{[4-(3,5-dichlorophenyl)-piperazine-1-carbonyl]-amino}-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 71.2%
m.p. : 210~212°C

¹H NMR(DMSO-d₆) : 1.25(3H,t), 2.83(2H,q), 3.30(4H,m), 3.66(4H,m), 4.03(3H,s), 4.53(2H,s), 5.41(1H,t), 6.63(1H,s), 6.79(3H,m), 7.11(2H,m), 7.23(1H,m), 7.42(1H,m), 7.55(4H,m), 7.71(1H,s), 8.09(2H,m), 8.32(1H,s), 9.74(1H,s)

Example 23

4-(3-fluorophenyl)-piperazine-1-carboxylic acid {5-[3-(acridine-9-yl-amino)-5-hydroxymethylphenylcarbamoyl]-6-ethyl-2-methoxy-pyridine-3-yl}amide.

The same reaction procedure to the example 17 18 were carried out using 2-ethyl-5-{{[4-(3-fluorophenyl)-piperazine-1-carbonyl]-amino}-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 72.1%
m.p. : 186~188°C

¹ H NMR(DMSO-d ₆)	:	1.25(3H,t), 4.04(3H,s), 6.69(2H,m), 7.63(4H,m),	2.84(2H,q), 4.55(2H,s), 7.22(4H,m), 8.17(2H,m),	3.28(4H,m), 5.39(1H,t), 7.33(1H,m), 8.37(1H,s),	3.67(4H,m), 6.63(2H,m), 7.44(1H,m), 9.66(1H,s)
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Please amend page 23, lines 9 and 25 as follows:

Example 24

4-(3-hydroxyphenyl)-piperazine-1-carboxylic acid {5-[3-(acridine-9-yl-amino)-5-hydroxymethylphenylcarbamoyl]-6-ethyl-2-methoxy-pyridine-3-yl} amide.

The same reaction procedure to the example 17 18 were carried out using 2-ethyl-5-{{[4-(3-hydroxyphenyl)-piperazine-1-carbonyl]-amino}-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 70.6%
m.p. : 196~198°C

¹ H NMR(DMSO-d ₆)	:	1.25(3H,t), 3.98(3H,s), 6.37(1H,s), 7.22(2H,m), 7.75(1H,m),	2.80(2H,q), 4.47(2H,s), 6.45(1H,d), 7.44(2H,m), 8.06(3H,m),	3.14(4H,m), 5.21(1H,t), 6.61(1H,m), 7.58(1H,m), 9.20(1H,s),	3.59(4H,m), 6.28(2H,m), 7.04(1H,t), 7.71(2H,m), 10.27(1H,s)
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Example 25

4-(3,4,5-trimethoxyphenyl)-piperazine-1-carboxylic acid {5-[3-(acridine-9-yl-amino)-5-hydroxymethylphenylcarbamoyl]-6-ethyl-2-methoxy-pyridine-3-yl} amide

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The same reaction procedure to the example 17 18 were carried out using 2-ethyl-5-{{[4-(3,4,5-trimethoxyphenyl)-piperazine-1-carbonyl]-amino}-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 66.8%
m.p. : 190~192°C

¹H NMR(DMSO-d₆) : 1.26(3H,t), 2.85(2H,q), 3.14(4H,m), 3.59(4H,m),
3.78(3H,s), 3.84(6H,s), 4.11(3H,s), 4.57(2H,s),
5.34(1H,t), 6.71(1H,s), 6.77(2H,s), 7.21(2H,s),
7.35(1H,m), 7.65(4H,m), 7.88(3H,m), 8.04(1H,s),
8.14(2H,m), 8.56(1H,s), 8.92(1H,s), 9.07(1H,s)

Please amend page 24, lines 10 and 24 as follows:

Example 26

N-(3-acridine-9-yl-amino)-5-hydroxymethylphenyl]-5-{{[4-(3,5-dimethoxyphenyl)-piperazine-1-carbothionyl]-amino}-2-ethyl-6-methoxynicotineamide.

The same reaction procedure to the example 17 18 were carried out using 5-{{[4-(3,5-dimethoxyphenyl)-piperazine-1-carbonyl]-amino}-2-methyl-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 69.8%
m.p. : 176~178°C

¹H NMR(DMSO-d₆) : 1.27(3H,t), 2.90(2H,q), 3.32(4H,m), 3.99(3H,s),
4.10(4H,m), 4.53(2H,s), 5.35(1H,s), 6.03(1H,s),
6.05(2H,d), 6.61(1H,s), 7.19(3H,m), 7.39(1H,m),
7.55(2H,m), 7.72(2H,m), 8.11(4H,m), 9.16(1H,s),

Example 27

N-(3-acridine-9-yl-amino)-5-hydroxymethylphenyl]-5-{{[4-(3,5-dimethylphenyl)-piperazine-1-carbothionyl]-amino}-2-ethyl-6-methoxynicotineamide.

The same reaction procedure to the example 17 18 were carried out using 5-{{[4-(3,5-dimethylphenyl)-piperazine-1-carbothionyl]-amino}-2-methyl-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 71.2%
m.p. : 170~172°C

¹H NMR(DMSO-d₆) : 1.28(3H,t), 2.27(6H,s), 2.90(2H,q), 3.28(4H,m), 3.99(3H,s), 4.11(4H,m), 4.55(2H,s), 5.39(1H,t), 6.54(3H,m), 6.70(1H,s), 7.15(2H,m), 7.32(1H,m), 7.47(1H,m), 7.60(2H,m), 7.76(2H,m), 8.02(1H,s), 8.13(2H,m), 8.42(1H,s), 9.70(1H,s)

Please amend page 25, lines 11 and 23 as follows:

Example 28

N-(3-acridine-9-yl-amino)-5-hydroxymethylphenyl]-5-{{[4-3-fluorophenyl)-piperazine-1-carbothionyl]-amino}-2-ethyl-6-methoxynicotineamide.

The same reaction procedure to the example 17 18 were carried out using 5-{{[4-(3-fluorophenyl)-piperazine-1-carbonyl]-amino}-2-methyl-6-methoxynicotinic acid and [3-(acridine-9-~~yl~~-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 70.8%
 m.p. : 176~178°C

¹H NMR(DMSO-d₆) : 1.26(3H,t), 2.87(2H,q), 3.36(4H,m), 3.94(3H,s),
 4.09(4H,m), 4.46(2H,s), 5.21(1H,t), 6.61(2H,m),
 6.82(2H,m), 7.26(4H,m), 7.46(1H,s), 7.66(3H,m),
 7.71(1H,s), 8.05(2H,m), 9.10(1H,s), 10.27(1H,s),

Example 29

N-(3-(acridine-9-yl-amino)-5-hydroxymethylphenyl]-5- {[4-3,5-dichlorophenyl]-piperazine-1-carbothionyl]-amino}-2-ethyl-6-methoxynicotineamide

The same reaction procedure to the example 17 18 were carried out using 5- {[4-(3,5-dichlorophenyl)-piperazine-1-carbothionyl]-amino-2-methyl-6-methoxynicotinic acid and [3-(acridine-9-yl-amino)-5-aminophenyl]-methanol to give the titled compound.

yield : 69.8%
 m.p. : 174~176°C

¹H NMR(DMSO-d₆) : 1.26(3H,t), 2.86(2H,q), 3.42(4H,m), 3.93(3H,s),
 4.07(4H,m), 4.47(2H,s), 5.2(1H,t), 6.54(1H,s),
 6.91(1H,s), 6.99(2H,m), 7.11(2H,m), 7.43(2H,s),
 7.58(3H,m), 7.72(2H,m), 8.03(2H,m), 9.09(1H,s)

Please amend page 27, lines 9 – 12 and 22 as follows:

Methods and results of the tests are as follows:

Experimental 1 : *In vitro* antitumor effect against human tumor cell lines.

A. Tumor cell lines : A549 (human non-small lung cell)

SKOV – 3 (human ovarian cell)

HCT-15 (human colon cell)

XF-498 (human CNS cell)

SKMEL-2 (human melanoma cell)

b. ~~5-10³-2-10⁴~~ $5 \times 10^3 \sim 2 \times 10^4$ cells were added into each well of 96-well plate and cultured in 5% CO₂ incubator at 37°C for 24 hours.

Please amend page 29, lines 2 and 19 as follows:

It was found that the compounds of the present invention have the even or superior antitumor activities ED₅₀(μ g/ml) than that of cisplatin, the control against human solid cancer cell lines.

TABLE 1.

ED₅₀(μ g/ml)

Ex. No.	A549	SK-OV-3	SK-MEL-2	XF-498	HCT-15
2	0.12	0.12	0.01	0.18	0.19
3	0.12	0.19	0.03	0.18	0.13
9	0.24	0.19~	0.15	0.15	0.15
16	0.08	0.14	0.02	0.09	0.07
19	0.21	0.17	0.18	0.38	0.27
Cisplatin	0.81	0.71	0.71	0.77	3.03

Experimental 2 : *In vitro* antitumor effects against animal leukemia cells.

B. Method : Dye Exclusion Assay.

1) The concentration of P388 cells being cultured in RPMI 1640 media containing 10% FBS was adjusted to ~~1-10⁶~~ 1×10^6 cells/ml.

2) Each sample drug of a concentration diluted in the ratio of log dose was added into cell culture media and cultured at 37 °C for 48 hours in 50% CO₂ incubator, and then viable cell number was measured by dye exclusion test using trypan blue.

3) The concentration of each sample compound showing 50% cell growth inhibition(IC₅₀) compared with the control was determined and listed in the table 2 below.

Please amend page 30, lines 10 and 16 as follows:

C. Results

As the result of measurement of antitumor activities IC₅₀(μ g/ml) against P388 mouse cancer cells of the compounds according to the present invention, it was found that the compounds tested have equal to or higher antitumor activities than those of the control drug, mitomycin C.

Table 2

Ex. No.	P388 ($\mu\text{g}/\text{ml}$)
2	0.3
3	1.0
4	0.9
9	0.4
16	0.3
Mitomycin C	1.1

Please amend page 31, lines 25 and 26 as follows:

Experimental 4 : Acute toxicity test (LD₅₀) :

a. Method : Litchfield-Wilcoxon method.

6-week-old ICR mice(male $30 \pm 2.0\text{g}$) were fed freely with solid feed and water at room temperature, $23 \pm 1^\circ\text{C}$ and at humidity $60 \pm 5\%$. Sample drugs were injected into the abdominal cavities of mice. Each group comprised 6 mice. Observed during 14 days, external appearances and life or death thereof were recorded, and also, visible lesions were observed from dead mice by dissection. LD₅₀ value was calculated by Litchfield-Wilcoxon method.

Please amend page 32, line 14 as follows:

{Industrial applicability}